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ECBC-TR-833

ANALYSIS OF MARINE BIOTA FOR CHEMICAL WARFARE MATERIALS BY MEANS OF A GAS CHROMATOGRAPH/MASS SPECTROMETER SYSTEM

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14. ABSTRACT
The U.S. Army Edgewood Chemical Biological Center's (ECBC) Directorate of Program Integration Environmental Monitoring Branch developed a procedure and conducted a Method Detection Limit (MDL) study for the analysis of Chemical Warfare Materials (CWM) in Fish Tissue in support of the Hawai'i Undersea Military Munitions Assessment (HUMMA) project. The scope of ECBC's study included developing new methodology to detect, accurately quantitate, and find the Limit of Quantitation for detecting the CWM Lewisite, Mustard, and it's breakdown products 1,4-Dithiane and 1,4-Thioxane; and extracting and analyzing samples collected in the HUMMA assessment area for CWM. Data from the MDL study and sample analysis are included.

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PREFACE

The work described in this report was authorized under Project No. 9VEV1, Environmental Monitoring Laboratory. This work was started in January 2009 and completed in September 2009.

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ANALYSIS OF MARINE BIOTA FOR CHEMICAL WARFARE MATERIALS BY MEANS OF A GAS CHROMATOGRAPH/MASS SPECTROMETER SYSTEM

1. INTRODUCTION

According to historical records, between the years 1933 and 1946 the United States Armed Forces disposed of chemical munitions and containers of bulk chemical agent (also referred to as chemical warfare materials or CWM) off O'ahu, Hawaii, according to the accepted maritime disposal procedures of the time. The specific chemical agents disposed include the blister agents Mustard (HD) and Lewisite (L). The Department of Defense (DoD) ended its practice of sea disposal (Figures 1 and 2) of military munitions and CWM in 1970 and disposal at sea was generally prohibited by Congress in 1972 with the passage of the Marine Protection, Research and Sanctuaries Act.

Figure 1. Disposal of Munitions at Sea (photo courtesy of the National Archives and Records Administration).

In 2008 the Environmental Monitoring Branch of the U.S. Army Edgewood Chemical and Biological Center (ECBC) was tasked by the Office of the Deputy Assistant Secretary of the Army for Environment, Safety and Occupational Health (ODASA-ESOH) to provide chemical agent safety and analytical support to the University of Hawaii at Manoa (UH). UH was subcontractor to Concurrent Technologies Corporation (CTC) under Task No.: 0496 of Contract W74V8H-04-D-0005 issued by the National Defense Center for Energy and Environment (NDCEE). The task for the Hawai'i Undersea Military Munitions Assessment (HUMMA) was to evaluate whether the munitions have the capability to significantly impact human health (specifically in regard to the introduction into the food chain for this report) and the environment. From a broader perspective, HUMMA's objective was to develop and demonstrate cost-efficient and effective methodologies for surveying and sampling historic munitions sea disposal sites.

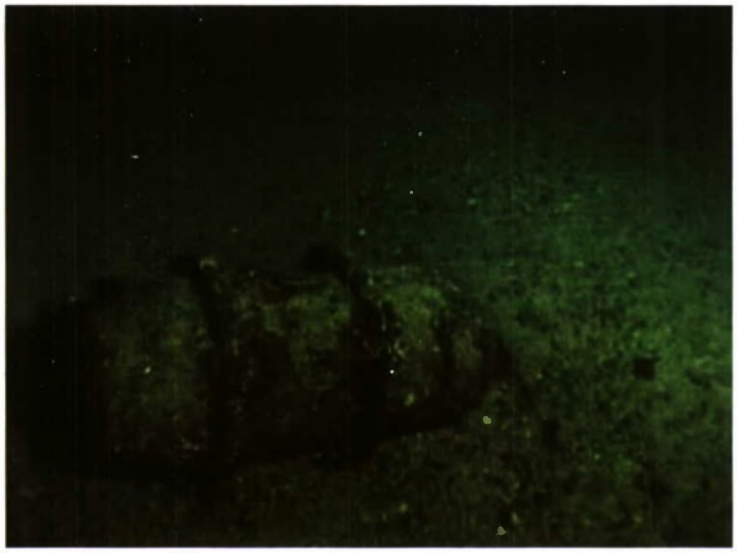

Figure 2. Modern Day Munition Located in the HUMMA Study Area, note similarity to items in Figure 1 (photo courtesy of Hawaii Undersea Researeh Laboratory).

In addition to providing on-site analysis of water and sediment, ECBC was tasked to provide a method of analysis to determine the levels (if any) of CWM (HD, Lewisite (L), and the HD break-down products 1,4-Dithiane and 1,4-Thioxane) in biota that would be harvested in the HUMMA Study Area. The experimental process, Method Detection Limit (MDL) study, and the analytical results for the samples collected in the spring and fall of 2009 aboard the Kilo Moana (Figure 3) are discussed herein.

Figure 3. University of Hawaii's (UH) Research Vessel (R/V) *Kilo Moana.*

2. EXPERIMENTAL PROCEDURES

2.1 Chemical Agents of Interest

Based on historical research on sea- disposal operations in Hawaii, HD was identified as possibly being present in the study area and although documentation did not indicate the presence of L to be likely, concern was expressed about possible impacts to human health and the environment if it had been disposed in the area. Thus, the chemical agents HD and Lewisite along with the HD breakdown products 1,4-Dithiane and 1,4-Thioxane identified as the compounds of potential concern for this marine biota tissue study.

2.1.1 Sulfur Mustard (HD)

Sulfur mustard (Figure 4), [bis(2-chloroethyl)sulfide], is a vesicant (blister agent) and alkylating agent, producing cytotoxic action on cell tissue. The rate of detoxification of HD in the body is very slow and repeated exposures produce a cumulative effect. Its toxic hazard is high for inhalation, ingestion and skin and eye absorption, but the most common acute hazard is from liquid contact with skin. HD sometimes smells like garlic, onions, or mustard and sometimes has no odor. It can be a vapor (the gaseous form of a liquid), an oily-textured liquid, or a solid.

HD has a relatively high melting point (57°F) and will usually form a solid mass at normal ocean temperatures at depth. HD is heavier than seawater (density is 1.27 g/cm^3 compared to 1.03 g/cm^3 for seawater) and has only slight solubility (U.S. Army, 2005). Mustard continuously dissolves from an exposed surface into the water, but at a slow rate and can remain stable for years in underwater zones where there is little current or turbulence. The relatively low solubility of HD in water results in slow dissolution and a relatively low overall rate of hydrolysis. In most circumstances, the rate of destruction by hydrolysis is assumed to be nearly the same as the rate of dissolution. As a result, no more than a few parts per million of the un-hydrolyzed mustard will be present in the overlying water at any given time (Epstein et al., 1973).

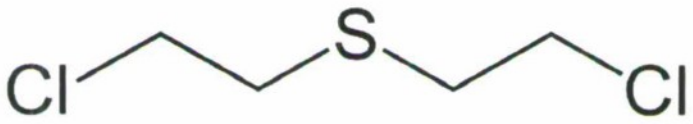

Figure 4. Chemical Structure of Sulfur Mustard (HD). Caution: HD is a potent vesicant and care must be taken to prevent exposure to liquid or vapor. It should only be manipulated by trained personnel using appropriate engineering controls and personal protective equipment.

2.1.2 Lewisite (L)

Lewisite (Chlorovinylarsine dichloride) is an extremely toxic arsenic containing blister agent that harms tissue and causes whole-body systemic effects. It is an oily, colorless liquid with an odor of geraniums. It has toxicity similar to HD; however, the effects are experienced immediately. Lewisite (Figure 5) hydrolyzes in water to form the toxic byproducts chlorovinyl arsenic acid (CVAA) and chlorovinyl arsenous oxide (CVAO) (Figure 6).

Lewisite has a significantly lower melting point (-18°C) than HD and will generally be found as a liquid at temperatures normal to ocean depths. The solubility of lewisite in seawater is not a significant factor in its fate and transport as hydrolysis is virtually instantaneous in water (Munro et al., 1999).

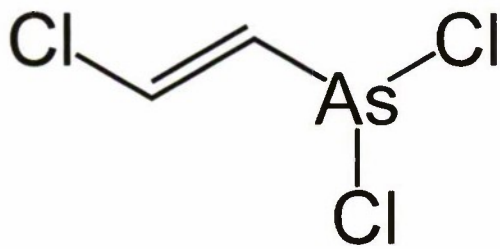

Figure 5. Chemical Structure of Lewisite. Caution: Lewisite is a potent vesicant and care must be taken to prevent exposure to liquid or vapor. It should only be manipulated by trained personnel using appropriate engineering controls and personal protective equipment.

Due to the fast hydrolysis of Lewisite into CVAA and CVAO a derivitization reaction must be performed prior to analysis. This reaction is carried out with β-Mercaptoethanol. As is illustrated in Figure 6, the Lewisite, CVAA and CVAO molecules are all derivitized into the target compound for GCMS analysis.

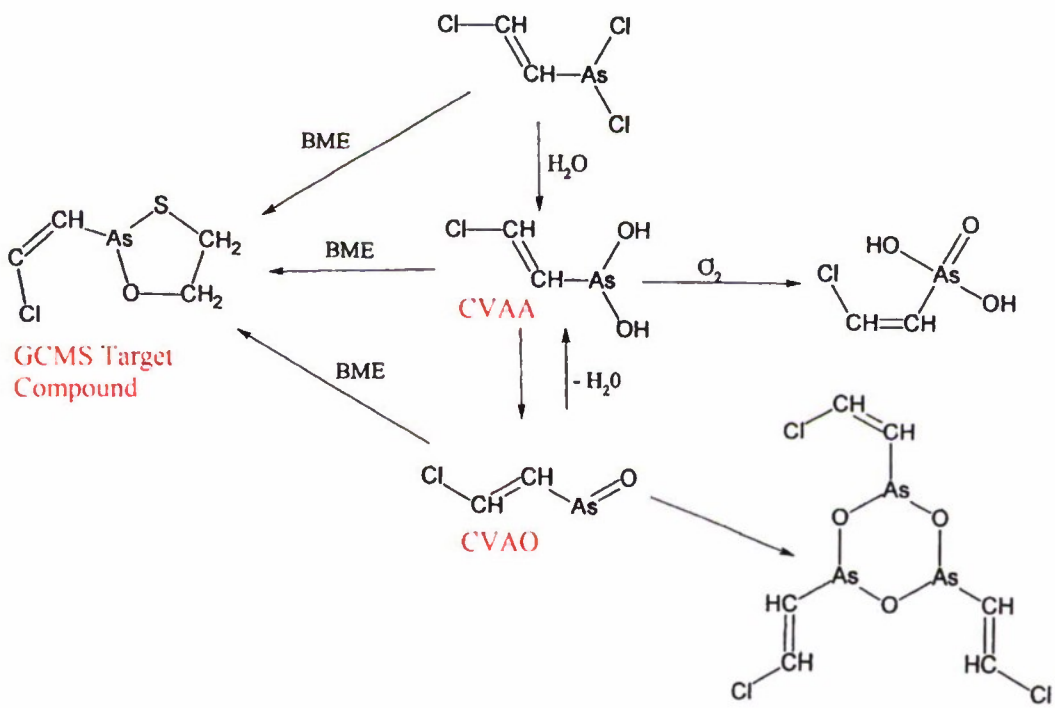

Figure 6. Lewisite Derivitization by β-Mercaptoethanol

2.1.3 1,4-Dithiane

1,4-Dithiane (Figure 7) is a mustard thermal breakdown product. It is a pale yellow powder in its natural state with an extremely unpleasant smell. Exposure may eause irritation to the eyes, respiratory system, and skin. Along with 1,4-Thoixane, 1,4-Dithiane is a readily GCMS identifiable breakdown produet that can be detected utilizing the same methodology as HD and Lewisite.

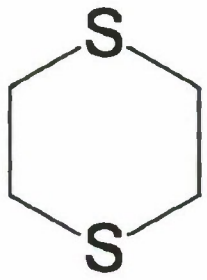

Figure 7. Chemical Structure of 1,4-Dithiane

2.1.4 1,4-Thioxane

1,4-Thioxane (Figure 8) is a mustard thermal breakdown produet. It is a flammable liquid and vapor. Exposure may eause central nervous system depression, eye and skin irritation, cardiae disturbanees, and respiratory and digestive tract irritation.

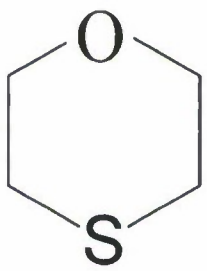

Figure 8. Chemieal Structure of 1,4-Thioxane

2.1.5 Near Drinking Water Level Laboratory Standards

The Environmental Monitoring Braneh reeeived stock solutions of Chemical Warfare Agents (CWA) from the Chemieal Transfer Facility (CTF) at the researeh, development, test and evaluation (RDTE) dilute level. It is neeessary for the stoek solutions to be diluted to a working eoneentration.

After receipt the stock solutions were volumetrically diluted to specific working levels that were used for extraction and instrument calibration. Two separate lots were received for each agent. The first was used to prepare calibration standards and the second to prepare an independent calibration verification standard. Preparations of these standards were conducted according to the procedures found in Standard Operating Procedure (SOP) CNG-048: Preparation of Near Drinking Water Level Chemical Agent Standards. For this study, the dilutes were prepared in dichloromethane (CH_2Cl_2).

2.2 Instrumentation

An Agilent 6890N GC with a 5973Mass Spectrometer (Figure 9) was used for analysis of the HUMMA samples.

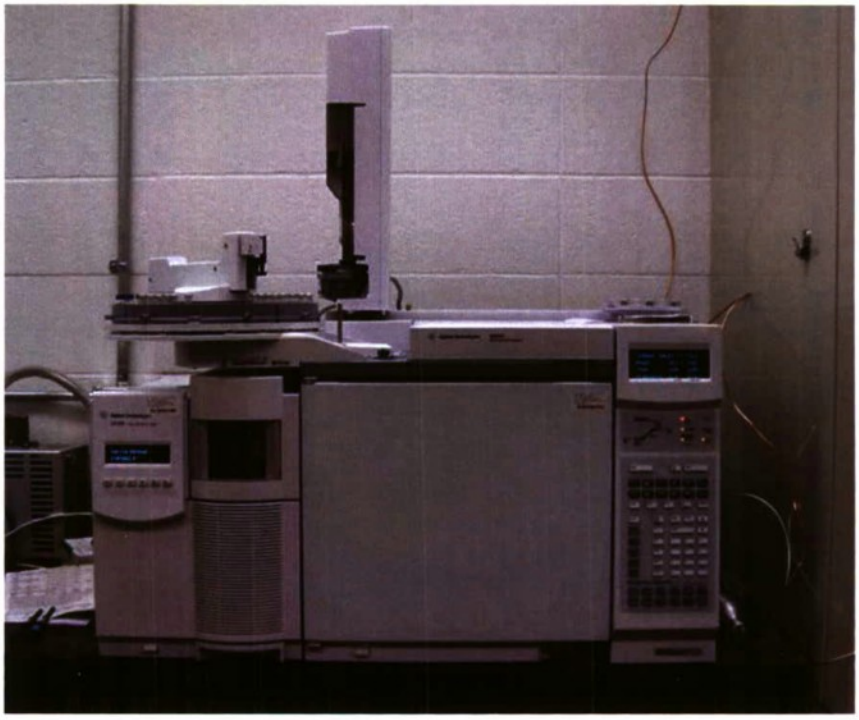

Figure 9. Gas Chromatograph/Mass Spectrometer

2.2.1 Gas Chromatograph (GC)

An analytical system, complete with a temperature programmable gas chromatograph (GC) suitable for splitless injection and all required accessories, including syringes, analytical columns, and gases, was used.

2.2.2 Capillary Column

A 30 m x 0.25 mm, ID 1 μm film thickness, silicone-coated fused silica capillary column (Agilent DB-1701 or equivalent) was used. The GC should be equipped with variable constant differential flow controllers so that the column flow rate will remain constant throughout desorption and temperature program operation.

2.2.3 Mass Spectrometer (MS)

A Mass Spectrometer capable of scanning from 35 to 300 amu every 2 seconds or less, using 70 V (nominal) electron energy in the electron impact ionization mode was used. The mass spectrometer must be capable of introducing a mass spectrum for 4-Bromofluorobenzene (BFB), which meets all select criteria when 1 - 50 ng of the GC/MS tuning standard (BFB) are injected into the GC and analyzed in the Selective Ion Mode (SIM). To ensure sufficient precision of mass spectral data, the desirable MS scan rate allows acquisition of at least ten spectra, whereas a sample component elutes from the GC.

2.2.4 GC/MS Interface

Any GC-to-MS interface may be used that gives acceptable calibration points at 2 ng or less per injection for each compound of interest and achieves acceptable tuning performance criteria. For a narrowbore capillary column, the interface is usually capillary-direct into the MS source.

2.2.5 Data System

A computer system was interfaced to the mass spectrometer. The system allowed for the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

The computer had software that can search any GC/MS data file for ions of a specific mass and that can plot such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Integrating the abundances in any EICP between specified time and scan-number limits is an important part of the data processing and was performed. The most recent version of the Environmental Protection Agency (EPA) / National Institute of Science and Technology (NIST) Mass Spectral Library was also used.

2.2.6 Calibration

For the MDL study and sample analysis a five point calibration curve ranging from 100 to 500 µg/L (10 to 50 µg/L for HD) was used. The calibration was verified at 250 µg/L (25 µg/L for HD).

2.3 Method Detection Limit (MDL) Study

The first item ECBC was tasked with was to determine the lowest possible point that the analytes could be detected, and the point they could practically be quantitated. To this end a MDL study was performed as outlined in the Department of Defense (DoD) Quality Systems Manual (QSM) for Environmental Laboratories Version 3 (May 2005).

2.3.1 Fish Tissue Sample Preparation

To imitate the fish sample matrix Onaga (Ruby Snapper), a whole frozen Red Snapper was purchased from a commercial supermarket. After thawing, the fillets were removed from the fish utilizing a filleting knife. The fillets were cut into pieces and then pulverized with a mortar and pestle into a single homogenous sample. Due to the similarity in sample matrix of fish tissue and shrimp tissue a separate MDL was not performed for the shrimp. This decision was supported by the results of the Matrix Spike / Matrix Spike Duplicate (MS/MSD) that showed adequate recovery with minimal interference.

2.3.2 Sample Extraction

Two grams of pulverized fillet was weighed into a 16mm test tube (Figure 10). Fifty microliters of each matrix spiking standard (5000 µg/L for 1,4-Dithiane, 1,4-Thioxane, and Lewisite; 500 µg/L for HD) and 100 µL of the surrogate/ internal standard spike Bromofluorobenzene / Hexachlorobenzene (BFB/HCB at 5000 µg/L each) was added to the test tube. For the Method Blank sample only the surrogate/internal standard was added. The tubes were refrigerated overnight at -4 °C (15 h), then removed from the fridge and allowed to come to room temperature. A total volume of 2 mL of Dichloromethane containing 0.1% β–mercaptoethanol (BME) was added to the test tube. The BME is used in excess to derivitize lewisite and its hydrolysis products CVAO and CVAA so that it can be analyzed by GCMS. The test tube was vortexed for approximately 1 min and then centrifuged at 5000 RPM for 10 min. The organic extract layer was then passed through a 0.4 µm Polytetrafluoroethylene (PTFE) filter into a 2mL vial for analysis. Samples were analyzed on a direct injection GCMS system according to the procedures specified in ECBC Internal Operating Procedure (IOP) MT-8 Revision 5: Analysis of Chemical Warfare Agents in Extracts using a Gas Chromatograph/Mass Spectrometer System.

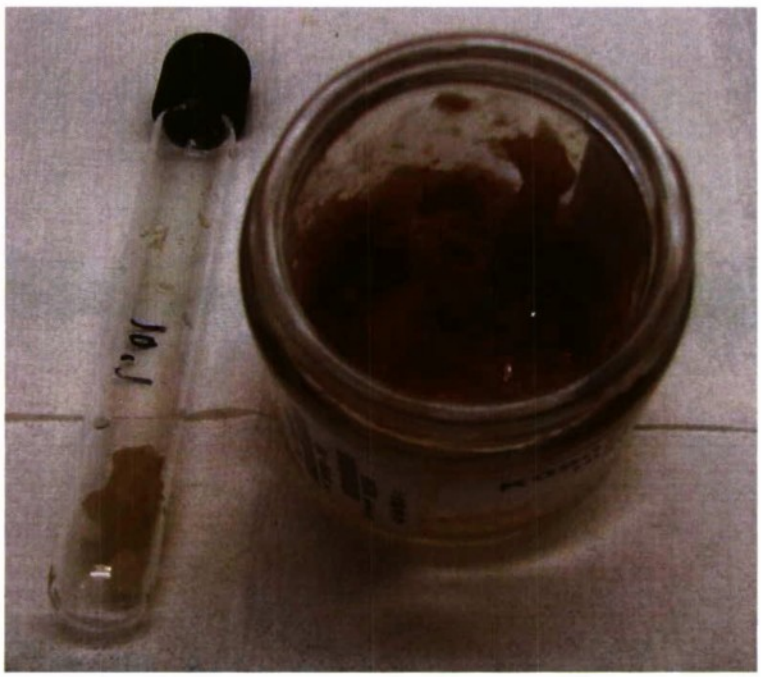

Figure 10. Fish Tissue Sample Received at ECBC. There is approximately 2 g in the test tube.

2.3.3 Analysis Parameters

The Target Concentration for 1,4-Thioxane; 1,4-Dithiane; and Lewisite was 125 µg /Kg. The target concentration for HD was 12.5 µg /Kg. Their characteristic ions in SIM are shown in Table 1.

Table 1. Charaeteristie Masses (M/Z) for Agents of Interest

CHARACTERISTIC IONS		
	Primary Characteristic Ion	Secondary Characteristic Ions
1,4-Dithianc	120	59, 61, 92
1,4-Thioxane	104	61, 74
Mustard (HD)	109	111, 158, 160
Lewisite (L) derivatized by BME	212	107, 151, 186

2.3.4 MDL Results

A successful MDL study was completed with all of the target analytes detected in the sample extract with the Method Blank clear of any analyte detection to ½ the Limit of Quantitation. The results are tabulated below in Tables 2 and 3.

Table 2. Instrument Results for MDL Extracts Analyzed by GC/MS

RAW DATA				
True Value of Spike: µg/Kg = ppb				
	1,4-Thioxane	**1,4-Dithiane**	**HD**	**Lewisite**
	125	125	12.5	125
Found Value of Spike: µg/Kg = ppb				
Replicate	**1,4-Thioxane**	**1,4-Dithiane**	**HD**	**L**
1	119	111	9	61
2	143	129	10	67
3	134	117	8	70
4	137	127	10	60
5	146	130	10	72
6	144	134	10	79
7	126	118	10	64
8	125	125	11	71
9	139	140	13	56
10	150	142	11	74
Mean	136.3	127.4	10.3	67.4
Standard Deviation	10.24	9.91	1.18	7.11
Bias (Accuracy)	109%	102%	82%	54%
Precision	7.5%	7.8%	11.4%	10.5%

Table 3. Calculated MDL and PQL Values

	Method Detection Limit (MDL) (µg/Kg)				Practical Quantitation Limit (PQL) (µg/Kg)			
	1,4-Thioxane	1,4-Dithiane	HD	L	1,4-Thioxane	1,4-Dithiane	HD	L
At the instrument	28.9	27.96	3.3	20.0	86.6	83.9	9.9	60.1
In the sample	28.9	27.96	3.3	20.0	86.6	83.9	9.9	60.1

The final reporting limit is the higher of the MDL and the Lowest Calibration Point. Based on this study, the following Limits of Quantitation (LOQ) were established for the analysis of fish tissue for CWM.

1,4-Thioxane, 1,4-Dithiane, Lewisite = 100 µg/Kg = ppb
Mustard (HD) = 10 µg/Kg = ppb

Samples with no detections above the MDL are reported as "Clear of agents of interest to the Limit of Quantitation." Any detection that falls above the MDL but below the LOQs are given a "J" value to indicate a degree of uncertainty in the result.

3. HUMMA SAMPLE ANALYSIS

3.1 HUMMA Sample Collection

From late April through early May 2009, sampling of fish and shrimp (Figures 11 and 12) in or near the HUMMA Study Area took place. The samples were packaged and shipped to ECBC for CWM analysis.

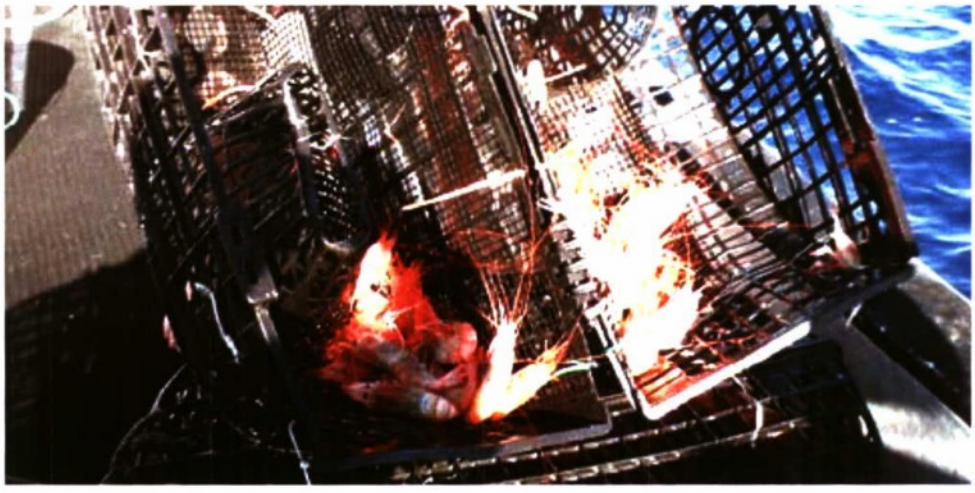

Figure 11. Shrimp Samples Caught in One of the Shrimp Traps Hauled During the Biota Collection.

The fish tissues samples analyzed included fillets only from a Large Onaga (see figure 11), whereas shrimp tissues analyzed included tails only, to be reflective of local consumption habits. Fish fillets were of sufficient mass to represent a unique sample; however, in some cases, two or more shrimp tails were combined to achieve the minimum sample mass required. In these instances, shrimp of roughly the same size and from the same shrimp trap were combined. Samples were sent directly to Columbia Analytical Services, where they were processed and analyzed for energetics and metals. Columbia Analytical Services also prepared a subset of extract that was sent to ECBC to be analyzed for HD, Lewisite and the degradation products 1,4-Dithiane and 1,4-Thioxane (The University of Hawaii at Manoa, 2010).

3.2 GC/MS Sample Analysis

For GCMS analysis the first step is to verify the tune. This must be done every 12 h. The tune is verified by introducing a mass spectrum for 4-Bromofluorobenzene (BFB), which meets select criteria when 1 – 50 ng of the GC/MS tuning standard (BFB) are injected into the GC and analyzed in the Selective Ion Mode (SIM). After the tune has been verified calibration can commence. After calibration is complete ($R^2 > 0.990$), an initial calibration verification is run. If the verification meets the QC limits, an analytical batch can then be analyzed.

Figure 12. Large Onaga (target species) Caught Onboard F/V *Red Raven* During the HUMMA Biota Sampling.

3.2.1 Analytical Batch Composition

Each Analytical Batch consisted of an Instrument Blank, Method Blank, Lab Control Standard, Lab Control Standard Duplicate, Matrix Spike, Matrix Spike Duplicate, and Samples (limited to 20 per batch).

3.2.1.1 Instrument Blank (IB)

An Instrument Blank was analyzed to demonstrate that interferences from the analytical system, glassware, and reagents are under control. All initial instrument blanks must be free of target analytes to one-half the Limit of Quantitation (LOQ) before analysis can commence. If analytes are

deteeted above this level thcn the souree of eontamination must be identified and removed prior to analysis.

3.2.1.2 Method Blank (MB)

The Method Blank assesses the preparation batch for possible eontamination during the preparation and processing steps. The Method Blank was proeessed along with and under the same conditions as the associated samples to include all steps of the analytieal procedure.

3.2.1.3 Lab Control Standard/Lab Control Standard Duplieate (LCS/LCSD)

Thc LCS evaluatcs the performance of the total analytical system, including all preparation and analysis steps. Results of each sample are compared to established criteria and, if found to be outside these eriteria, indieates that the analytical system is "out of eontrol." When the results of the matrix spike analysis indicate a potential problem bccause of the sample matrix itself, the LCS/LCSD results are used to verify that the laboratory can perform the analysis in a elean matrix. For the HUMMA samplc analysis, tissue from a commercially purchased Red Snapper was used as the sample matrix for the LCS/LCSD.

3.2.1.4 Matrix Spike/Matrix Spike Duplicate (MS/MSD)

The MS indicates the effect of the sample matrix on the precision and accuracy of the results. The information from thcse controls is sample/matrix specific and not used to determine the validity of the entire batch. The MS and MSD must be the same matrix as the samples being analyzed in the batch. They are analyzed to answer the question "If the analyte is present in the sample matrix, ean the instrument deteet it?" For this HUMMA analysis one of the reeeived samples was used for the MS/MSD in eaeh batch that was analyzed.

3.2.1.5 Samples

The fish tissue and shrimp samples from HUMMA arrived at ECBC in May and September of 2009. They were received frozen and already pulverized and were stored at -16 °C prior to extraction. They were extracted according to the procedures detailed in the MDL study along with full batch Quality Control (QC). They were analyzed, reviewed, and final reports were sent out to the HUMMA project distribution list.

3.2.1.6 Continuing Calibration Verification (CCV)

The initial ealibration eurvc for each compound of interest was verified not less than once every 12 h prior to sample analysis using the introduction technique used for samples. This was aeeomplished by analyzing a Continuing Calibration Verification (CCV) standard at a concentration near the midpoint concentration for the ealibrating range of the GC/MS.

3.2.2 Data Analysis

Examples of Quantitation Reports (including Extracted Ion Chromatograms (EIC's) for 1,4-Dithiane, 1,4-Thioxane, HD, and Lewisite) can be found in the Appendix. A CCV, Sample Matrix Spike Sample, and a Samplc are shown. The three reports show a standard run at a known concentration, the matrix spiked to a known eoneentration and an un-spikcd sample.

3.3 HUMMA Sample Extraction

The samples were removed from the freezer and allowed to come to room temperature. For the MB, LCS, LCSD (see section 3.2.1 for description), 2 g of commercially purchased Red Snapper (prepared for extraction the same way as in the MDL study) was weighed inside of a 16mm test tube (Figure 12). For the MS, MSD (see section 3.2.1 for description), and Samples, 2 g of the pulverized fillet/shrimp received from HUMMA was weighed inside of a 16mm test tube. 100 µL of the surrogate/ internal standard spike Bromofluorobenzene / Hexachlorobenzene (BFB/HCB at 5000 µg/L each) was added to each tube. 100 µL of each matrix spiking standard (5000 µg/L for1,4-Dithiane, 1,4-Thioxane, and Lewisite; 500 µg/L for HD) were added to the LCS, LCSD, MS, and MSD. The 1.9 mL of 2 mL of Dichloromethane containing 0.1% β-mercaptoethanol (BME) was added to the test tube (1.7mL for the LCS, LCSD, MS, MSD). The BME is used in excess to derivitize lewisite and its hydrolysis products CVAO and CVAA, so that it can be analyzed by GCMS. The test tubes were vortexed for approximately 1 min and then centrifuged at 5000 RPM for 10 min. The organic extract layer was then filtered through a 0.4 µm PTFFTE filter into a 2mL vial for analysis. Samples were analyzed on a direct injection GCMS system according to the procedures specified in ECBC IOP MT-8 Revision 5: Analysis of Chemical Warfare Agents in Extracts using a Gas Chromatograph/Mass Spectrometer System.

3.4 Final Results

A total of forty eight tissue samples were received, extracted, and analyzed in support of the HUMMA project (the results are in Tables 4 and 5). All of the samples were analyzed in SIM. By comparing the EIC's (See Appendix) of the MS (for sample EML 091524) and Sample EML091524 it can be concluded that:

- First, there were no agents detected above the LOQ, and
- Second, the instrument would be capable of detecting the agents if they were in the sample. The remaining samples were all clear for HD, 1,4-Dithiane, and 1,4-Thioxane to the Laboratory LOQ.

Table 4. Sample Results for HUMMA Shrimp Samples

ECBC Sample Number	HUMMA Sample Number	Results (µg/Kg = ppb)			
		HD	Lewisite	1,4-Dithiane	1,4-Thioxane
EML091524	HUM001S	< 10	<100	<100	<100
EML091525	HUM002S	< 10	<100	<100	<100
EML091526	HUM003S	< 10	<100	<100	<100
EML091527	HUM004S	< 10	<100	<100	<100
EML091528	HUM005S	< 10	<100	<100	<100
EML091529	HUM006S	< 10	<100	<100	<100
EML091530	HUM007S	< 10	<100	<100	<100
EML091531	HUM008S	< 10	<100	<100	<100
EML091532	HUM009S	< 10	<100	<100	<100
EML091533	HUM010S	< 10	<100	<100	<100
EML091534	HUM011S	< 10	<100	<100	<100
EML091535	HUM012S	< 10	<100	<100	<100
EML091536	HUM013S	< 10	<100	<100	<100
EML091537	HUM014S	< 10	<100	<100	<100
EML091538	HUM018S	< 10	<100	<100	<100
EML091539	HUM019S	< 10	<100	<100	<100
EML091540	HUM021S	< 10	<100	<100	<100
EML091541	HUM024S	< 10	<100	<100	<100
EML091542	HUM025S	< 10	<100	<100	<100
EML091543	HUM029S	< 10	<100	<100	<100
EML091544	HUM030S	< 10	<100	<100	<100
EML093424	HUM015S	< 10	<100	<100	<100
EML093425	HUM016S	< 10	<100	<100	<100
EML093426	HUM017S	< 10	<100	<100	<100
EML093427	HUM020S	< 10	<100	<100	<100
EML093428	HUM022S	< 10	<100	<100	<100
EML093429	HUM023S	< 10	<100	<100	<100
EML093430	HUM026S	< 10	<100	<100	<100
EML093434	HUM027S	< 10	<100	<100	<100
EML093432	HUM028S	< 10	<100	<100	<100

Note: The following Limits of Quantitation (LOQ) Apply: 1,4-Thioxane, 1,4-Dithiane, Lewisite = 100 µg/Kg
Mustard (HD) = 10 µg/Kg

Table 5. Sample Results for HUMMA Fish Tissue Samples

ECBC Sample Number	HUMMA Sample Number	Results (µg/Kg = ppb)			
		HD	Lewisite	1,4-Dithiane	1,4-Thioxane
EML091545	HUM001F	< 10	<100	<100	<100
EML091546	HUM002F	< 10	<100	<100	<100
EML091547	HUM003F	< 10	<100	<100	<100
EML091548	HUM004F	< 10	<100	<100	<100
EML091549	HUM005F	< 10	<100	<100	<100
EML091550	HUM006F	< 10	<100	<100	<100
EML091551	HUM007F	< 10	<100	<100	<100
EML091552	HUM008F	< 10	<100	<100	<100
EML091553	HUM009F	< 10	<100	<100	<100
EML091554	HUM010F	< 10	<100	<100	<100
EML091555	HUM011F	< 10	<100	<100	<100
EML091556	HUM012F	< 10	<100	<100	<100
EML091557	HUM013F	< 10	<100	<100	<100
EML091558	HUM014F	< 10	<100	<100	<100
EML091559	HUM015F	< 10	<100	<100	<100
EML091560	HUM016F	< 10	<100	<100	<100
EML091561	HUM017F	< 10	<100	<100	<100
EML091562	HUM018F	< 10	<100	<100	<100

Note: The following Limits of Quantitation (LOQ)Apply: 1,4-Thioxane, 1,4-Dithiane, Lewisite = 100 µg/Kg Mustard (HD) = 10 µg/Kg

4. DISCUSSION

ECBC was tasked with developing an analytical approach to detecting Chemical Warfare Material (CWM) and agent breakdown products in fish tissue. Our laboratory was able to develop a method to detect and accurately quantify the amount of 1,4-Dithiane, 1,4-Thioxane, Mustard and Lewisite in fish tissue utilizing Gas Chromatography / Mass Spectroscopy.

ECBC was able to analyze, validate, and generate results utilizing the procedures developed in the method detection limit study. The data generated during the project was used to determine if the target chemical agents and breakdown products were present below the Limit of Quantitation of 100 ppb for 1,4-Dithiane, 1,4-Thioxane, and L, and 10 ppb for HD. This analysis demonstrates that there was no direct contamination present in the biota samples collected; however, any fish metabolization of agent cannot be determined from this method.

This study will serve as the basis for any future ECBC efforts to extract and analyze biota samples in support of similar projects.

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REFERENCES

1. U.S. Army. Potential Military Chemical/Biological Agents and Compounds. FM 3-11.9, MCRP 3-37.1B, NTRP 3-11.32, and AFTTP(I) 3-2.55. January.

2. Epstein, J.; Rosenblatt, D.H.; Gallacio, A.; McTeague, W.F. Summary Report on a Data-base for Predicting Consequences of Chemical Disposal Operations. EASP 1200-12; 1973; AD-B955399.

3. Munro, N.B.; Talmage, S.S.; Griffin, G.D.; Waters, L.C.; Watson, A.P.; King, J.F.; Hauschild, V. The Sources, Fate, and Toxicity of Chemical Warfare Agent Degradation Products. *Environmental Health Perspectives* 1999, *107* (12) 933-974.

4. The University of Hawaii at Manoa in Association with Environet, Inc. Hawai'I Undersea Military Munitions Assessment (HUMMA) Draft Investigation Report for Hawaii -05; January 2009.

5. Environmental Data Quality Workgroup Department of Navy, Lead Service. Department of Defense (DoD) Quality Systems Manual (QSM) for Environmental Laboratories Version 3, May 2005.

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GLOSSARY

Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is > 0, and is determined from analysis of a sample in a given matrix containing the analyte. The Appendix contains the necessary equations for calculating method detection limits. (40 CFR part 136, Appendix B, rev. 1.11)

Practical Quantitation Limit (PQL) is a quantitation limit that represents a practical and routinely achievable quantitation limit with a high degree of certainty (>99.9% confidence) in the results.

Limit of Quantitation (LOQ) or lower limit of quantitation (LOQ) is the level above which quantitative results may be obtained with a specified degree of confidence. The LOQ is mathematically defined as equal to 10 times the standard deviation of the results for a series of replicates used to determine a justifiable limit of detection.

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ACRONYMS

amu	Atomic Mass Unit
BFB	Bromo Fluoro Benzene
BME	Beta Metcapto Ethanol
CCV	Continuing Calibration Verification
CTC	Concurrent Technologies Corporation
CTF	Chemical Transfer Facility
CVAA	Chlorovinyl Arsenic Acid
CVAO	Chlorovinyl Arsenic Oxide
CWM	Chemical Warfare Material
DoD	Department of Defense
ECBC	Edgewood Chemical and Biological Center
EIC	Extracted Ion Chromatogram
EICP	Extracted Ion Current Profile
EML	Environmental Monitoring Lab
EPA	Environmental Protection Agency
GC	Gas Chromatograph
HD	Sulfur Mustard
HUMMA	Hawai'i Undersea Military Munitions Assessment
IB	Instrument Blank
ICV	Initial Calibration Verification
IOP	Internal Operating Procedure
L	Lewisite
LCS/LCSD	Lab Control Standard / Lab Control Standard Duplicate
LOQ	Limit of Quantitation
MB	Method Blank
MDL	Method Detection Limit
MS	Mass Spectroscopy / Spectrometer
MS/MSD	Matrix Spike / Matrix Spike Duplicate
m	Meter
mL	Milliliter
mm	Millimeter
M/Z	Mass to Charge Ratio
ng	Nanogram
NDCEE	National Defense Center for Energy and Environment
NIST	National Institute of Science and Technology
ODASA-ESOH	Office of the Deputy Assistant Secretary of the Army for Environment, Safety and Occupational Health
PQL	Practical Quantitation Limit
ppb	Parts Per Billion
PTFE	Polytetrafluorocthylene
QC	Quality Control
QSM	Quality Systems Manual
RDT&E	Research, Development, Test and Evaluation
SIM	Selected Ion Mode

SOP	Standard Operating Procedure
UH	University of Hawaii
µg/L	Microgram Per Liter
µg/Kg	Microgram per Kilogram
µm	Micrometer

APPENDIX

INSTRUMENT QUANTITATION REPORTS FROM SAMPLE ANALYSIS

Quantitation Report (Not Reviewed)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270003.D
Acq On : 27 May 2009 7:47 am
Operator : BED
Sample : 250ug/L CCV
Misc : VI-56-3-Q
ALS Vial : 3 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 28 17:06:01 2009
Quant Method : C:\msdchem\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)
1) HCB	9.604	284	623396	250.00	ug/L	0.00
System Monitoring Compounds						
2) BFB	4.881	174	560034	251.51	ug/L	0.00
Spiked Amount 250.000	Range 60 - 134		Recovery = 100.60%			
Target Compounds						Qvalue
3) GB	4.448	99	1036191	287.28	ug/L	100
4) 1,4-THIOXANE	4.685	104	416853	256.65	ug/L	96
5) GD-1	6.036	99	272477	284.76	ug/L	99
6) GD-2	6.075	99	238967	291.59	ug/L	98
7) 1,4-DITHIANE	6.159	120	587811	244.31	ug/L	98
8) HN1	6.685	122	239637	225.28	ug/L	97
9) GA	7.010	106	92515	274.88	ug/L	97
10) HD	7.075	109	64603	27.66	ug/L	98
11) GF	7.258	99	1136262	313.42	ug/L	99
12) L	7.992	212	90634	308.52	ug/L #	57
13) HN3	8.532	156	521337	264.68	ug/L	99
14) VX	9.848	114	489637	331.09	ug/L	98

(#) = qualifier out of range (m) = manual integration (+) = signals summed

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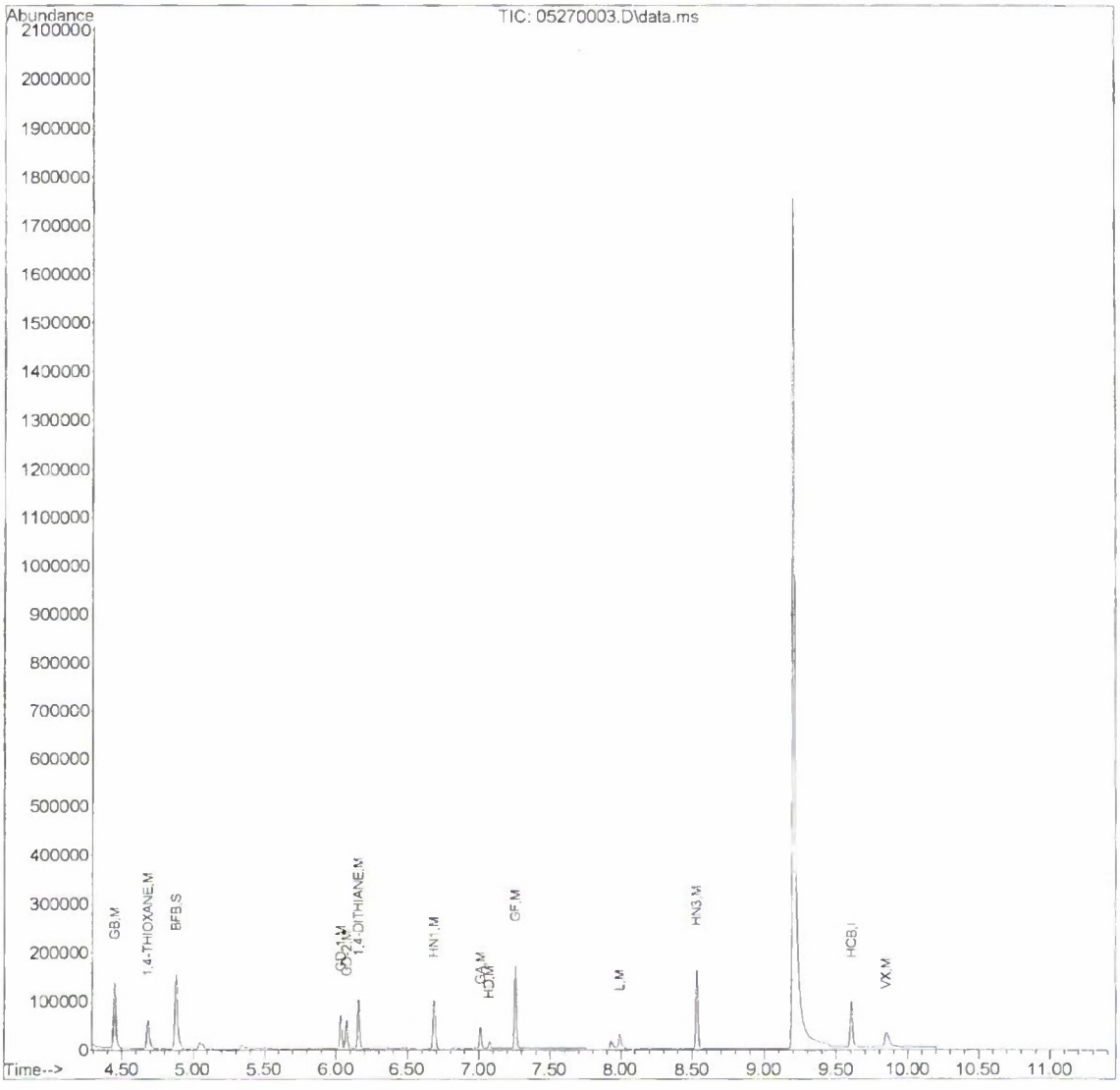
Figure 1. Quantitation Report for the Continuing Calibration Standard. Target concentration is 25 µg/L for HD and 250 µg/L for all other agents including Bromofluorobenzene (BFB) surrogate.

Quantitation Report (Not Reviewed)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270003.D
Acq On : 27 May 2009 7:47 am
Operator : BED
Sample : 250ug/L CCV
Misc : VI-56-3-Q
ALS Vial : 3 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 28 17:06:01 2009
Quant Method : C:\msdchem\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration



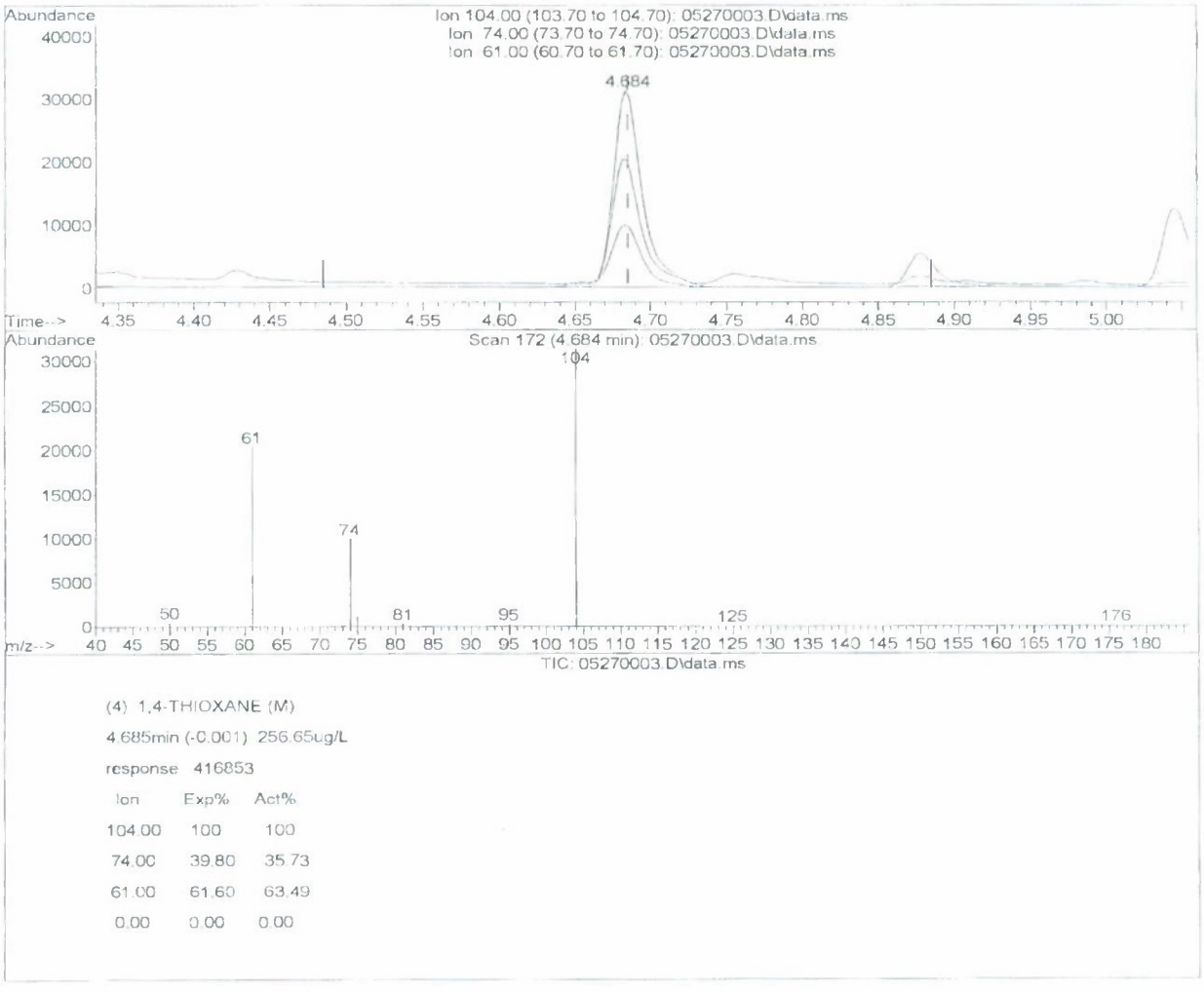
CWM.M Mon Mar 08 09:08:50 2010 Page: 2

Figure 2. Continuing Calibration Verification (CCV) Chromatogram. This spectrum shows the peaks and their retention times for the run.

Quantitation Report (Qedit)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270003.D
Acq On : 27 May 2009 7:47 am
Operator : BED
Sample : 250ug/L CCV
Misc : VI-56-3-Q
ALS Vial : 3 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 28 17:06:01 2009
Quant Method : C:\msdchem\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration



(4) 1,4-THIOXANE (M)

4.685min (-0.001) 256.65ug/L

response 416853

Ion	Exp%	Act%
104.00	100	100
74.00	39.80	35.73
61.00	61.60	63.49
0.00	0.00	0.00

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Figure 3. 1,4-Thioxane CCV Extracted Ion Chromatogram (EIC). The spectrum shows the peak and retention time produced when the characteristic ions for 1,4-Thioxane are extracted from the sample chromatogram and verified against the calibration for retention time and expected ion ratios.

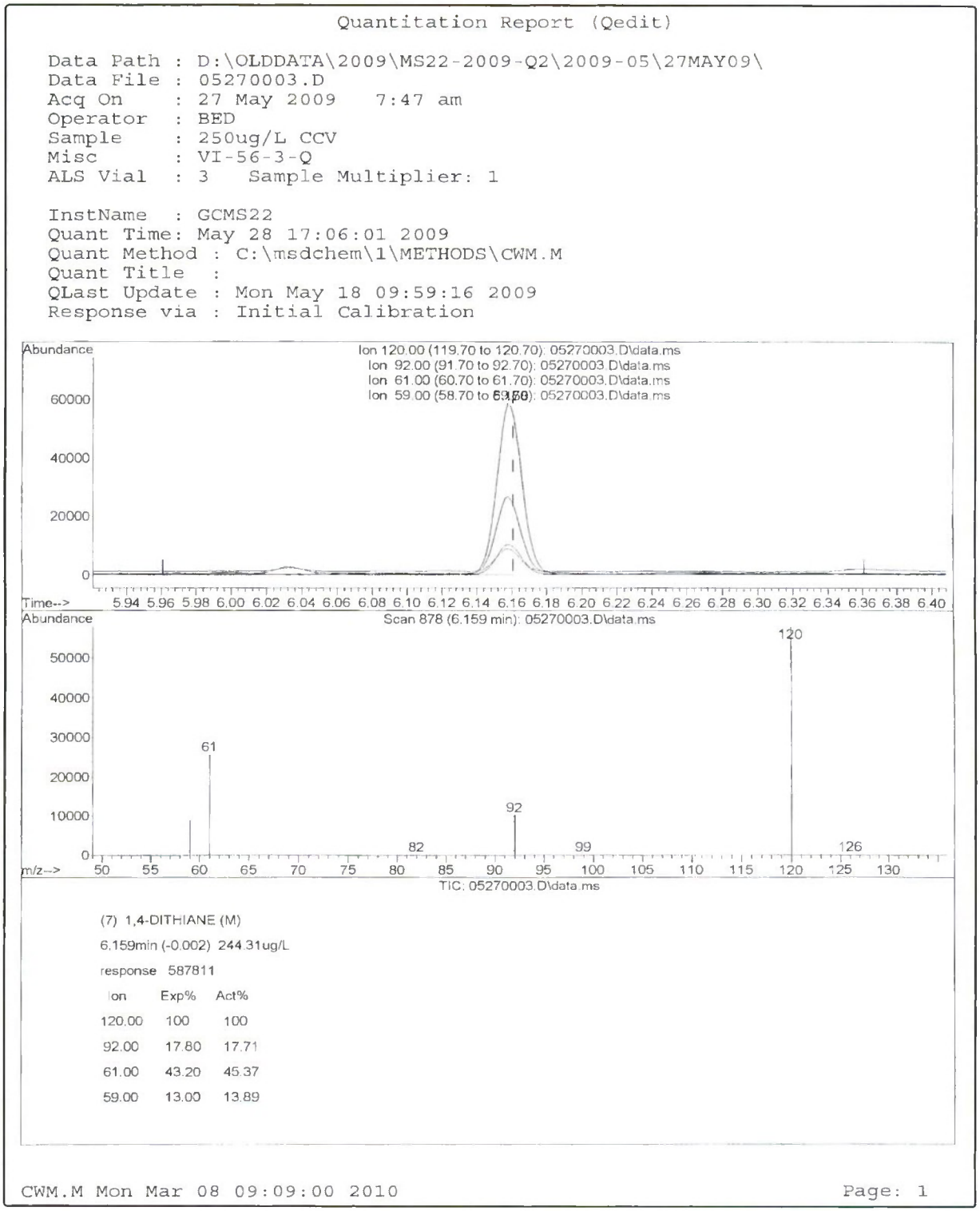


Figure 4. 1,4-Dithiane CCV Extracted Ion Chromatogram (EIC). This spectrum show the peak and retention time produced when the characteristic ions for 1,4-Dithiane are extracted from the sample chromatogram and verified against the calibration for retention time and expected ion ratios.

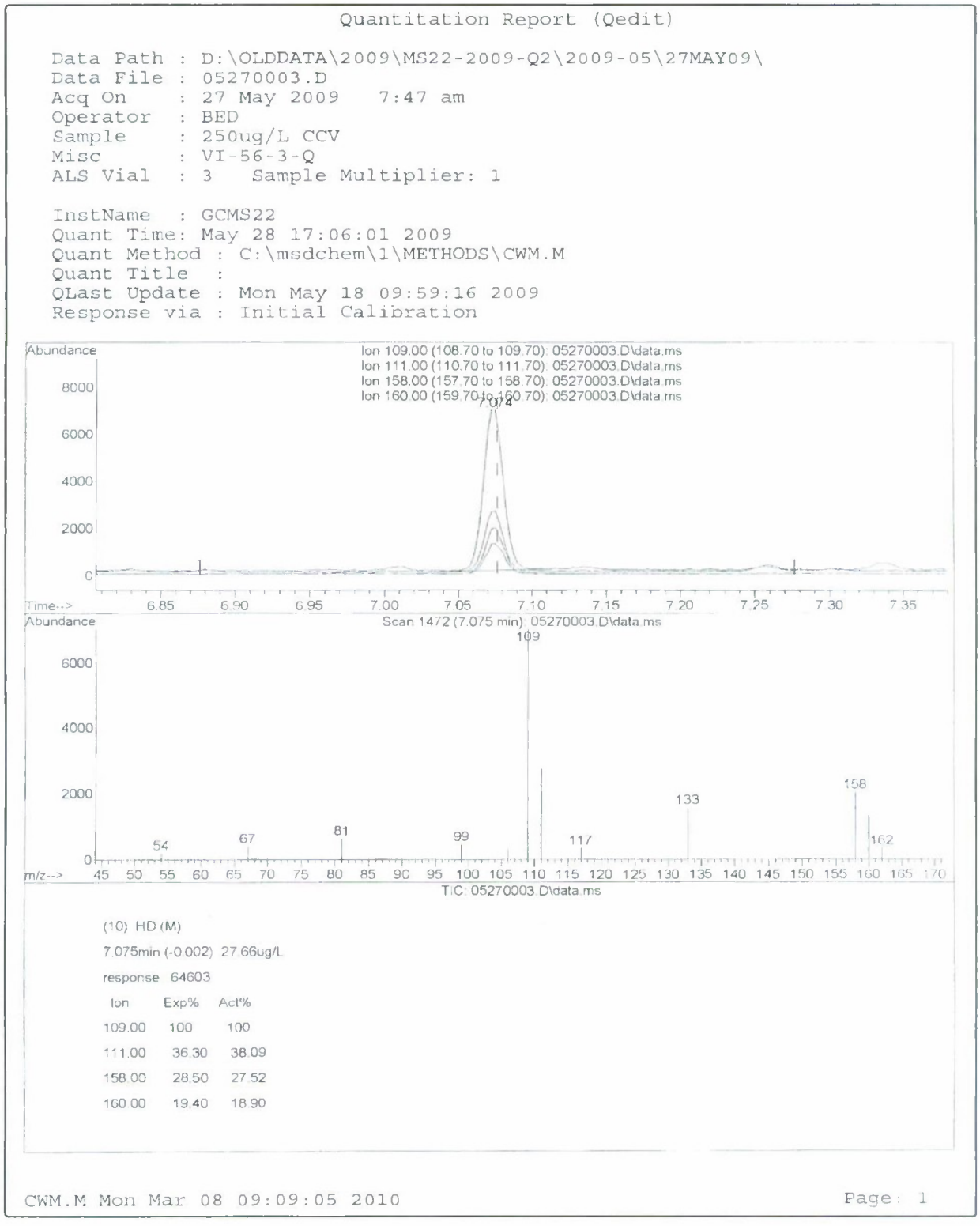


Figure 5. Sulfur Mustard (HD) CCV Extracted Ion Chromatogram (EIC). This spectrum shows the peak and retention time produced when the characteristic ions for HD are extracted from the sample chromatogram and verified against the calibration for retention time and expected ion ratios.

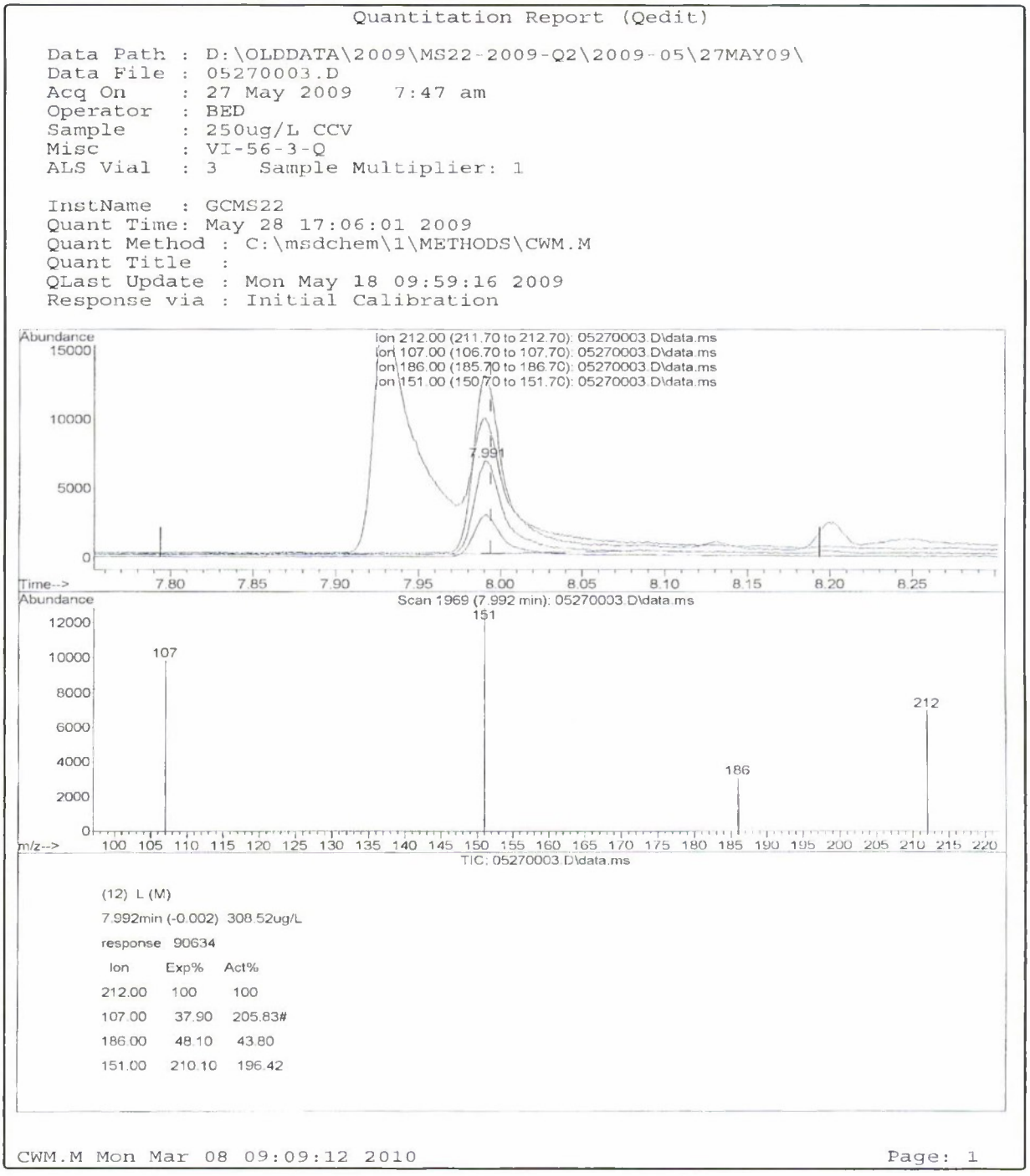


Figure 6. Lewisite CCV Extracted Ion Chromatogram (EIC). This spectrum shows the peak and retention time produced when the characteristic ions for HD are extracted from the sample chromatogram and verified against the calibration for retention time and expected ion ratios.

Quantitation Report (QT Reviewed)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270007.D
Acq On : 27 May 2009 9:11 am
Operator : BED
Sample : EML091524-MS
Misc : 09052602, FISH TISSUE
ALS Vial : 7 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 27 08:27:04 2009
Quant Method : C:\MSDCHEM\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)
1) HCB	9.603	284	671900	250.00	ug/L	0.00
System Monitoring Compounds						
2) BFB	4.880	174	616655	257.05	ug/L	0.00
Spiked Amount 250.000	Range 60 - 134		Recovery = 102.82%			
Target Compounds						Qvalue
3) GB	4.448	99	1028824	264.70	ug/L	99
4) 1,4-THIOXANE	4.684	104	429174	244.47	ug/L #	93
5) GD-1	6.036	99	286950	278.27	ug/L	99
6) GD-2	6.075	99	249573	282.67	ug/L	98
7) 1,4-DITHIANE	6.160	120	630612	243.09	ug/L	97
8) HN1	6.686	122	249193	217.46	ug/L	98
9) GA	7.011	106	95120	262.25	ug/L	97
10) HD	7.075	109	65565	26.02	ug/L	97
11) GF	7.258	99	1184436	303.61	ug/L	99
12) L	7.991	212	63503	213.42	ug/L #	61
13) HN3	8.532	156	521911	246.12	ug/L	99
14) VX	9.825	114	520759	327.23	ug/L	98

(#) = qualifier out of range (m) = manual integration (+) = signals summed

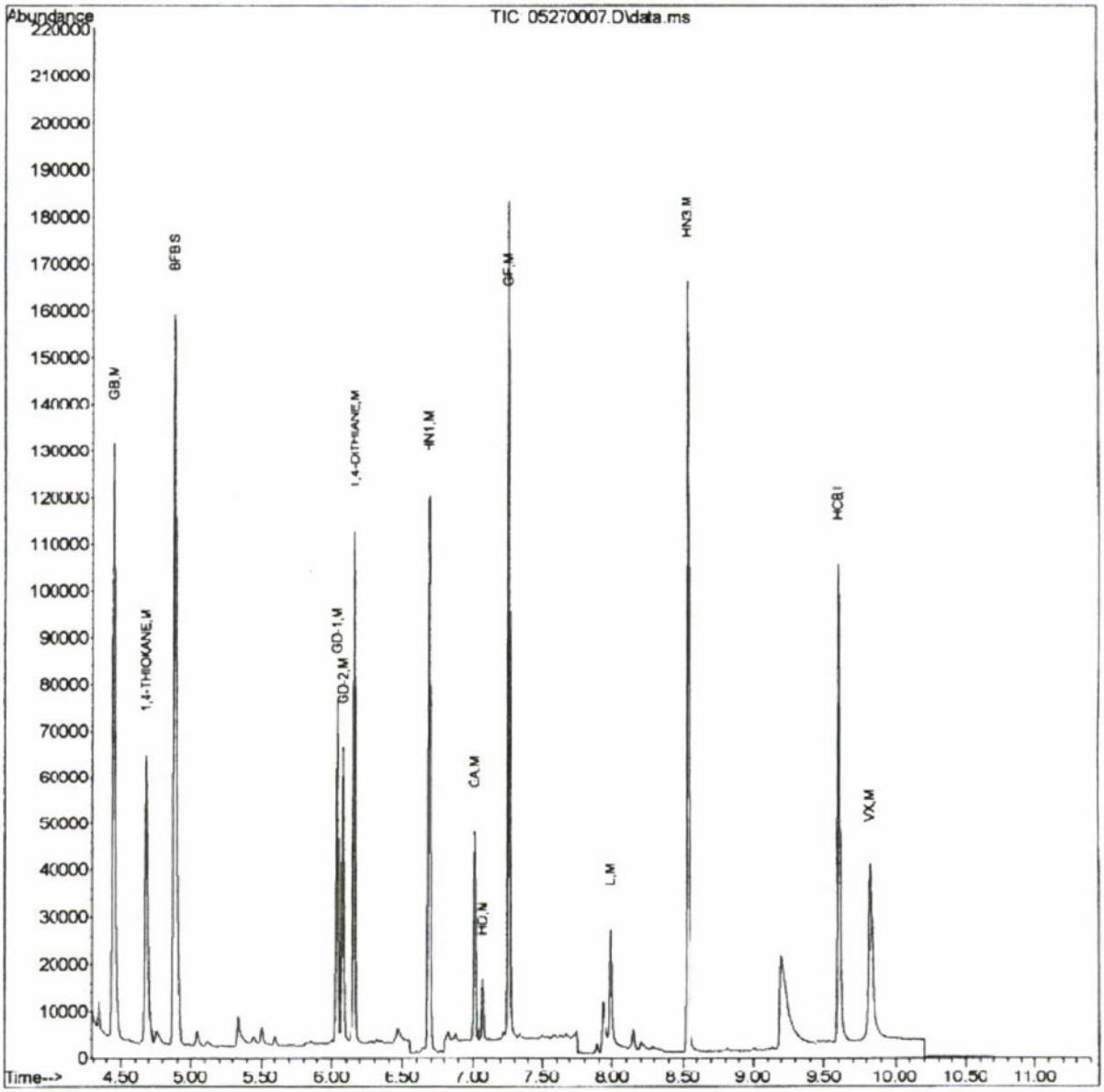
CWM.M Mon Mar 08 09:09:46 2010 Page: 1

Figure 7. Quantitation Report for the Matrix Spike Sample. One fish tissue sample was spiked with a known concentration of standard and taken through the extraction process to determine whether the agents of interest could be detected in the sample matrix. Target concentration is 25 µg/L for HD and 250 µg/L for all other agents.

Quantitation Report (QT Reviewed)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270007.D
Acq On : 27 May 2009 9:11 am
Operator : BED
Sample : EML091524-MS
Misc : 09052602, FISH TISSUE
ALS Vial : 7 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 27 08:27:04 2009
Quant Method : C:\MSDCHEM\1\METHODS\CWN.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration



CWN.M Mon Mar 08 09:09:45 2010 Page: 2

Figure 8. Matrix Spike Sample Chromatogram. The speetrum shows the peaks and their deteetion times for the Matrix Spike.

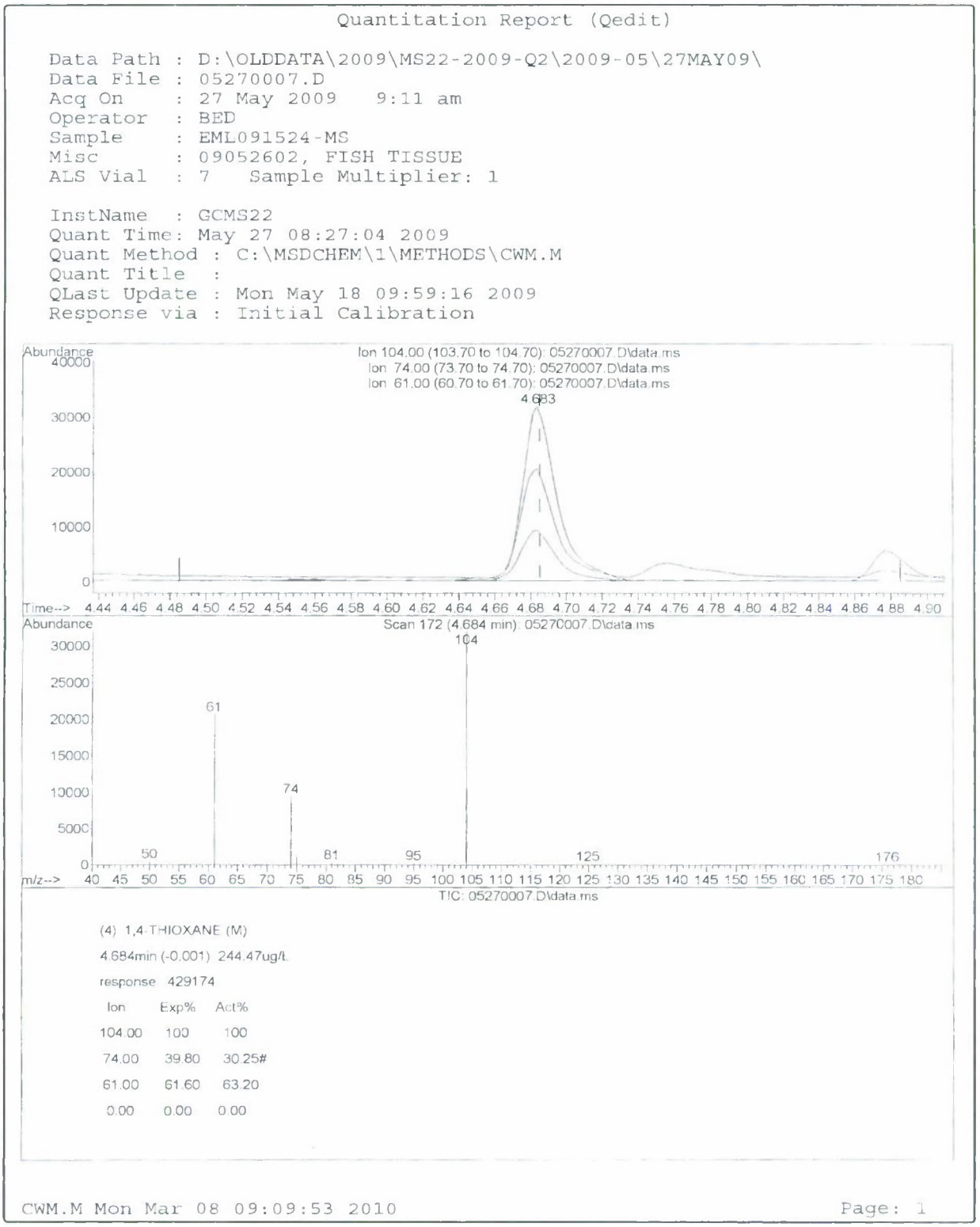


Figure 9. 1,4-Thioxane Matrix Spike Extracted Ion Chromatogram (EIC). The spectrum shows the peak and retention time produced when the characteristic ions for 1,4-Thioxane are extracted from the sample chromatogram and verified against the calibration for retention time and expected ion ratios.

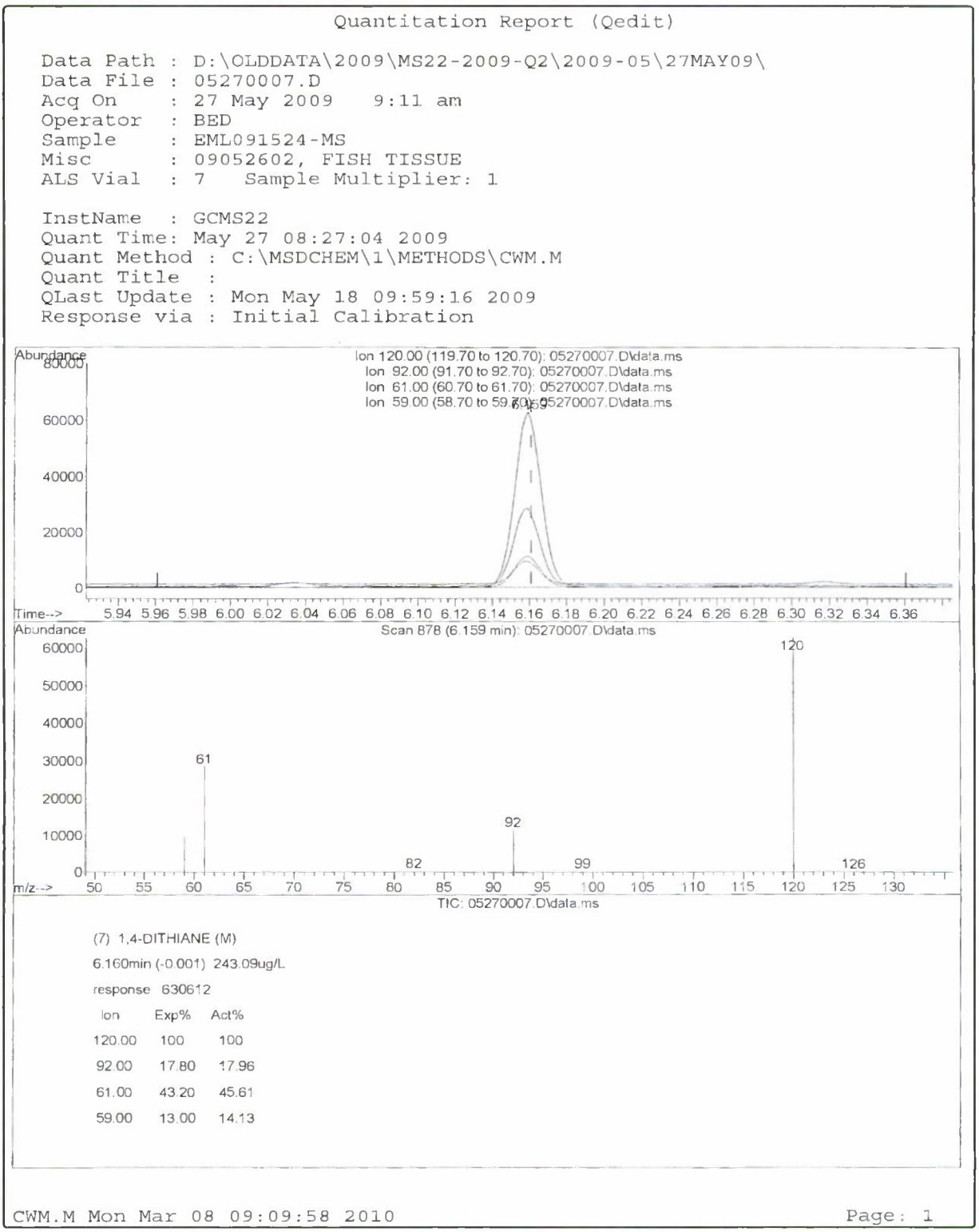
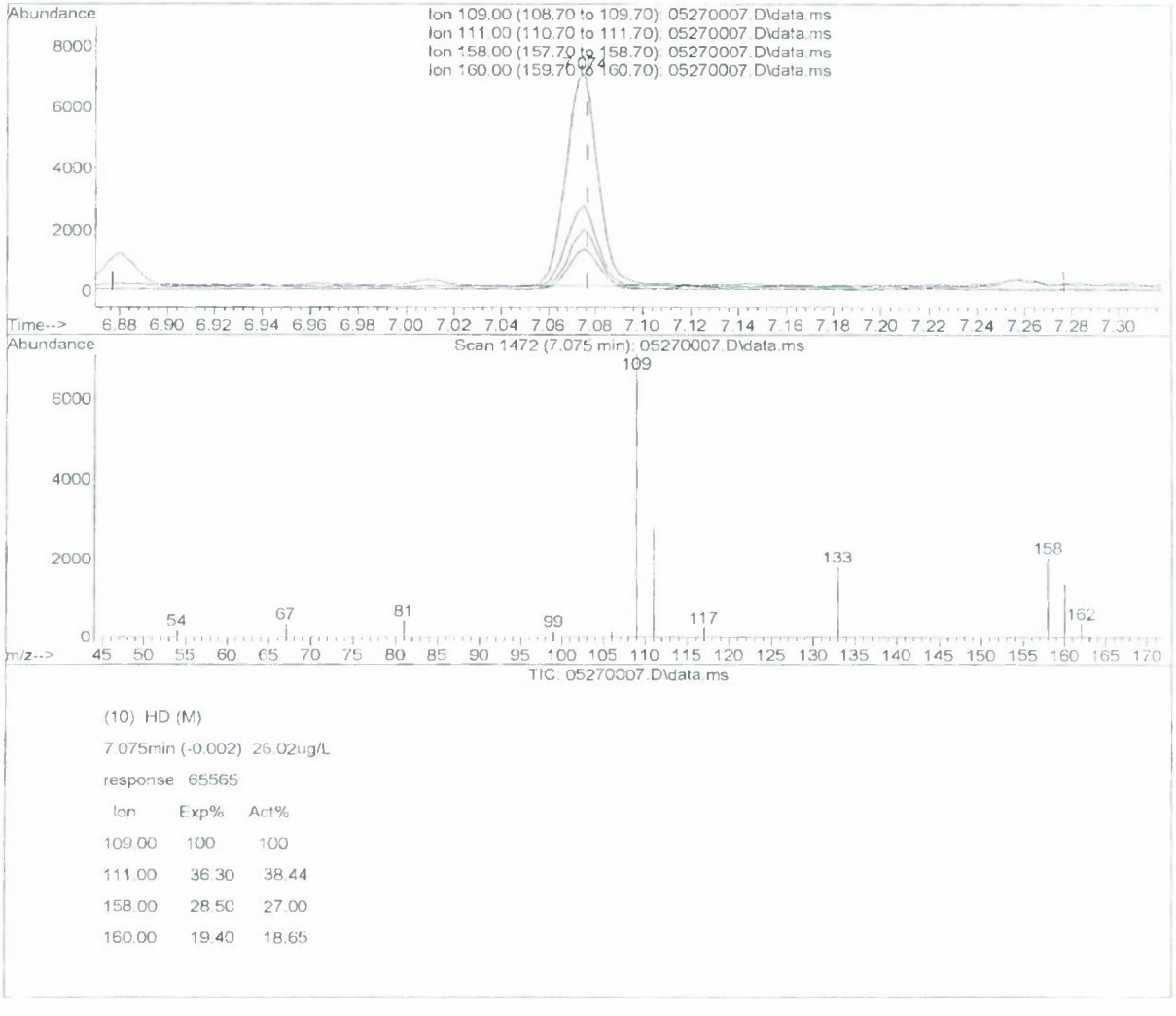


Figure 10. 1,4-Dithiane Matrix Spike Extracted Ion Chromatogram (EIC). The spectrum shows the peak and retention time produced when the characteristic ions for 1,4-dithiane are extracted from the sample chromatogram and verified against the calibration for retention time and expected ion ratios.

Quantitation Report (Qedit)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270007.D
Acq On : 27 May 2009 9:11 am
Operator : BED
Sample : EML091524-MS
Misc : 09052602, FISH TISSUE
ALS Vial : 7 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 27 08:27:04 2009
Quant Method : C:\MSDCHEM\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration



(10) HD (M)

7.075min (-0.002) 26.02ug/L

response 65565

Ion	Exp%	Act%
109.00	100	100
111.00	36.30	38.44
158.00	28.50	27.00
160.00	19.40	18.65

CWM.M Mon Mar 08 09:10:02 2010 Page: 1

Figure 11. Sulfur Mustard (HD) Matrix Spike Extracted Ion Chromatogram (EIC). The spectrum shows the peak and retention time produced when the characteristic ions for HD are extracted from the sample chromatogram and verified against the calibration for retention time and expected ion ratios.

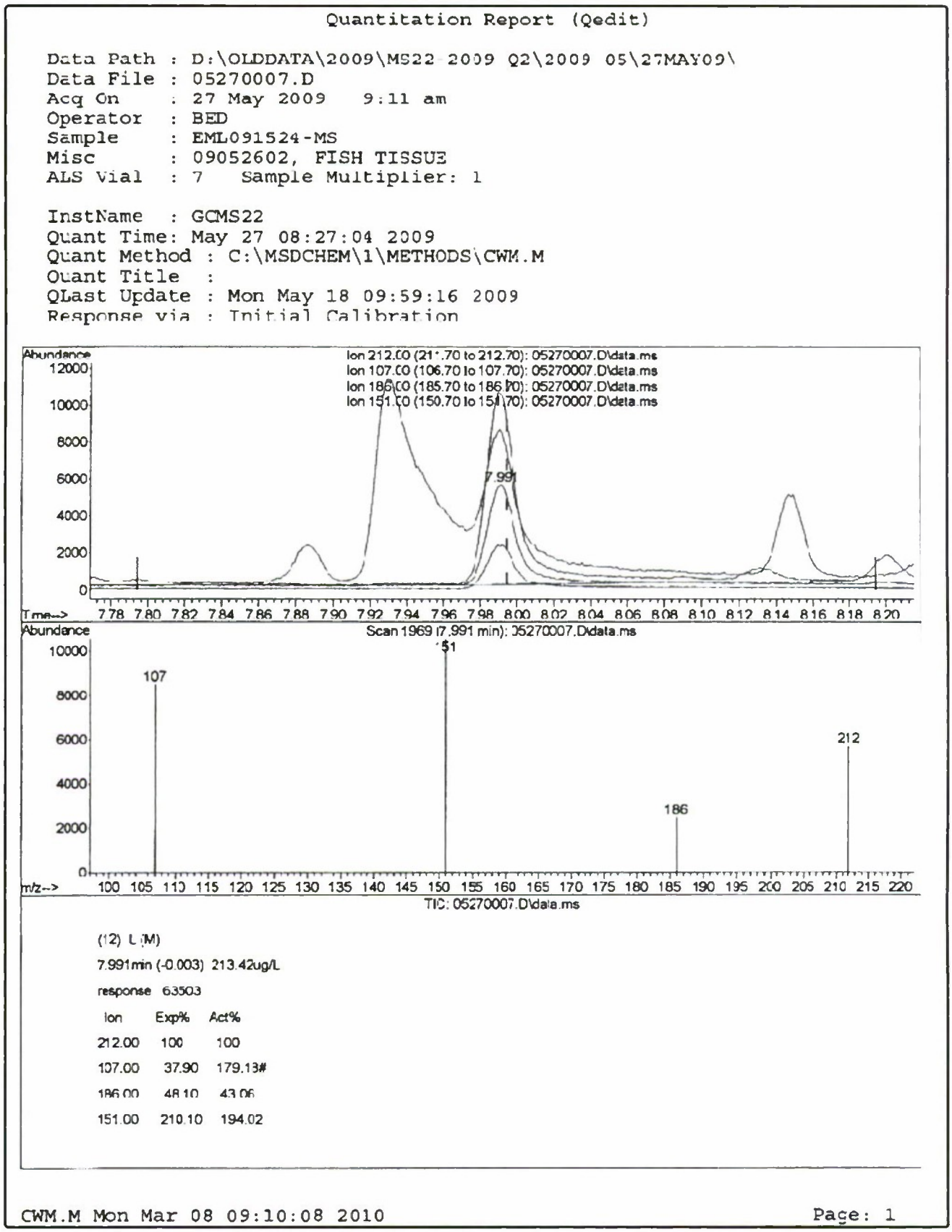


Figure 12. Lewisite Matrix Spike Extracted Ion Chromatogram (EIC). The spectrum shows the peak and retention time produced when the characteristic ions for HD are extracted from the sample chromatogram and verified against the calibration for retention time and expected ion ratios.

Quantitation Report (QT Reviewed)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270010.D
Acq On : 27 May 2009 10:14 am
Operator : BED
Sample : EML091524
Misc : 09052602, FISH TISSUE
ALS Vial : 9 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 27 10:08:48 2009
Quant Method : C:\MSDCHEM\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration

Internal Standards	R.T.	QIon	Response	Conc Units	Dev(Min)
1) HCB	9.603	284	599480	250.00 ug/L	0.00
System Monitoring Compounds					
2) BFB	4.880	174	557036	260.31 ug/L	0.00
Spiked Amount 250.000	Range 60 - 134		Recovery = 104.12%		
Target Compounds					Qvalue
3) GB	0.000	99	0	N.D.	
4) 1,4-THIOXANE	0.000	104	0	N.D.	
5) GD-1	0.000	99	0	N.D.	
6) GD-2	0.000	99	0	N.D. d	
7) 1,4-DITHIANE	0.000	120	0	N.D. d	
8) HN1	0.000	122	0	N.D.	
9) GA	0.000	106	0	N.D.	
10) HD	0.000	109	0	N.D.	
11) GF	0.000	99	0	N.D.	
12) I	0.000	212	0	N.D.	
13) HN3	0.000	156	0	N.D.	
14) VX	0.000	114	0	N.D.	

(#) = qualifier out of range (m) = manual integration (+) = signals summed

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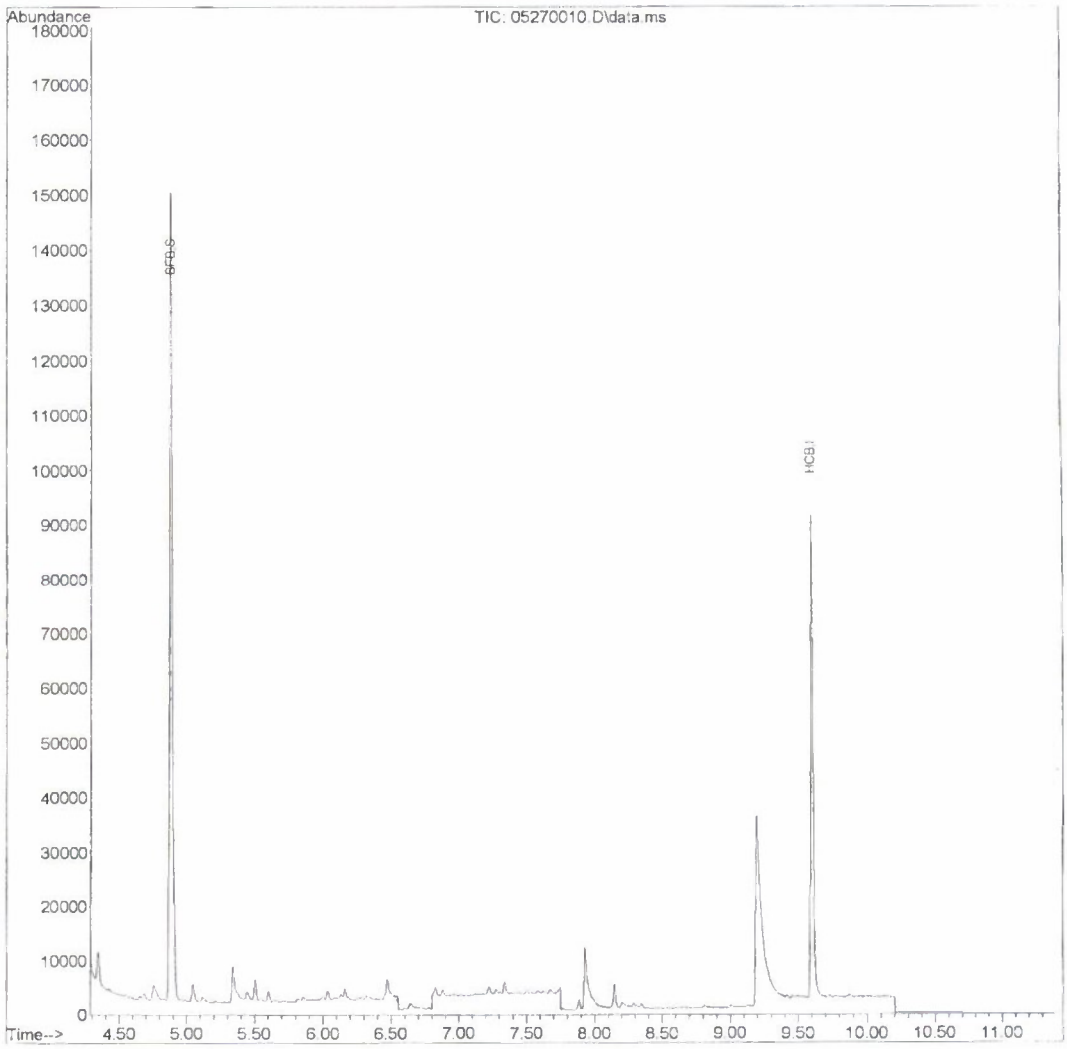
Figure 13. Quantitation Report for Sample EML091524 (HUM001S). The spectrum shows that there were no agents of interest detected. The Bromofluoro Benzene (BFB) surrogate recovery was 104%.

Quantitation Report (QT Reviewed)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270010.D
Acq On : 27 May 2009 10:14 am
Operator : BED
Sample : EML091524
Misc : 09052602, FISH TISSUE
ALS Vial : 9 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 27 10:08:48 2009
Quant Method : C:\MSDCHEM\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration



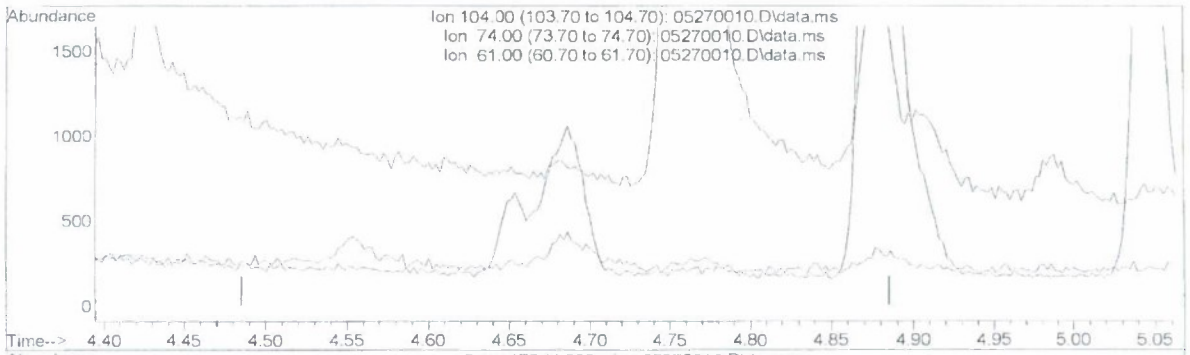
CWM.M Wed Mar 10 09:58:07 2010 Page: 2

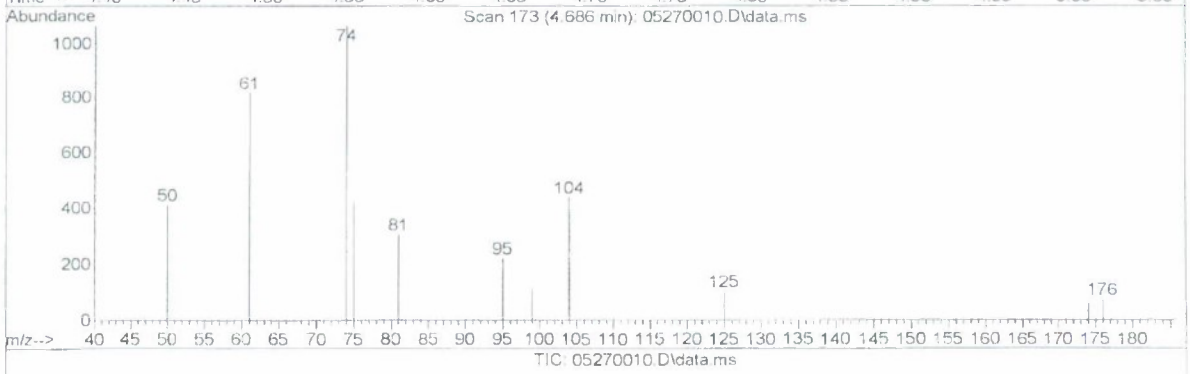
Figure 14. Sample EML091524 (HUM001S) Chromatogram. The spectrum shows only the Hexachlorobenzene (HCB) internal standard and BFB surrogate are present in the sample extract.

Quantitation Report (Qedit)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270010.D
Acq On : 27 May 2009 10:14 am
Operator : BED
Sample : EML091524
Misc : 09052602, FISH TISSUE
ALS Vial : 9 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 27 10:08:48 2009
Quant Method : C:\MSDCHEM\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration





TIC: 05270010.D\data.ms

(4) 1,4-THIOXANE (M)

4.686min (-4.686) 0.00ug/L

response 0

Ion	Exp%	Act%
104.00	100	0.00
74.00	39.80	0.00#
61.00	61.60	0.00#
0.00	0.00	0.00

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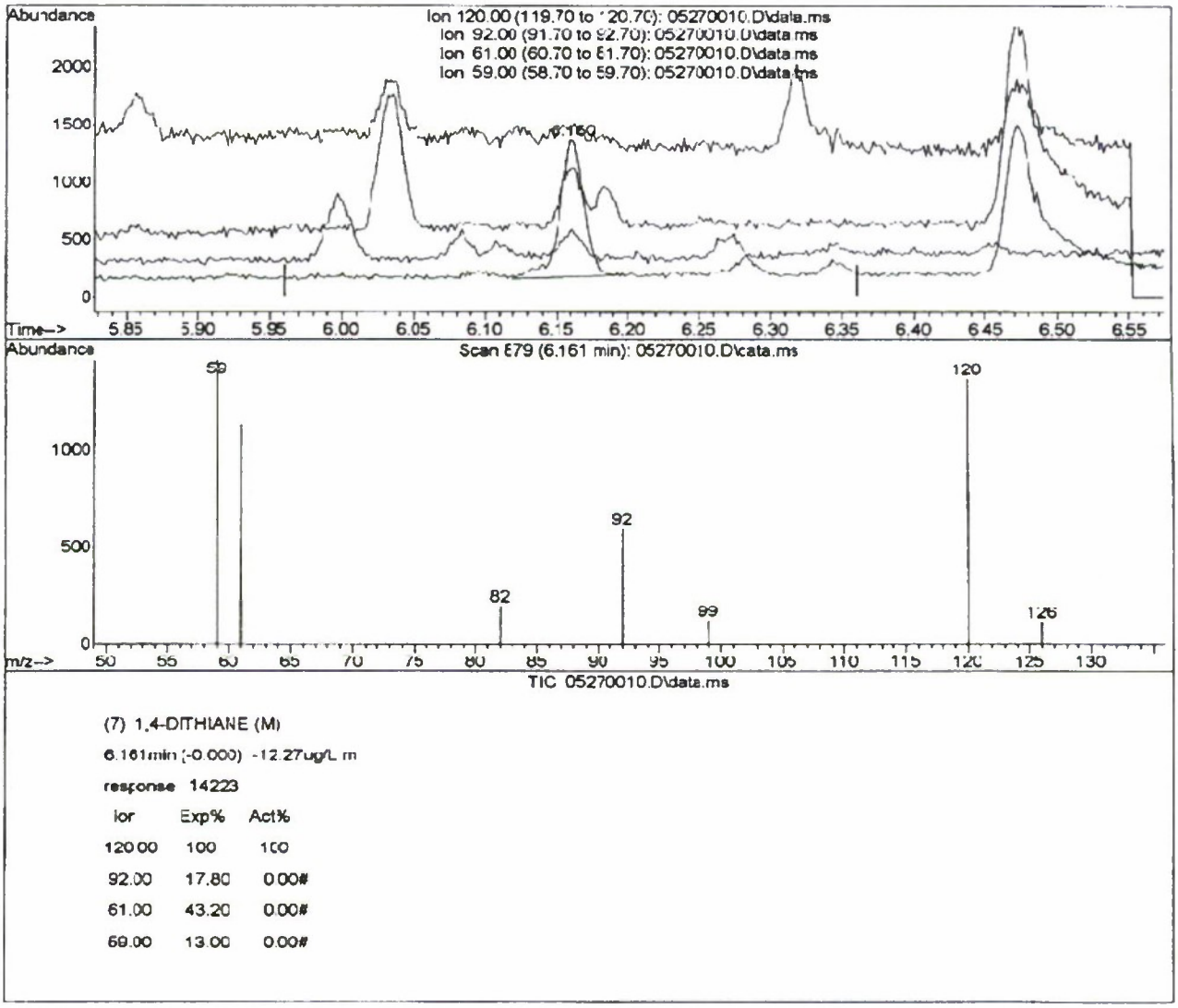
Figure 15. 1,4-Thioxane Extracted Ion Chromatogram (EIC) for Sample EML091524 (HUM001S). The spectrum shows no peaks present at the expected retention time of 4.686 min for the characteristic ions. Per the IOP, this sample is considered to be clear for 1,4-Thioxane to the Limit of Quantitation.

Quantitation Report (Qedit)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270010.D
Acq On : 27 May 2009 10:14 am
Operator : BED
Sample : EML091524
Misc : 09052602, FISH TISSUE
ALS Vial : 9 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 27 10:08:48 2009
Quant Method : C:\MSDCHEM\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration



CWM.M Wed Mar 10 11:29:56 2010 Page: 1

Figure 16. 1,4-Dithiane Extracted Ion Chromatogram (EIC) for Sample EML091524 (HUM001S). The spectrum shows that even when the minor peaks present at the 1,4-Dithiane retention time of 6.161 min are integrated the amount is a negative value. Per the IOP, this sample is considered to be clear for 1,4-Dithiane to the Limit of Quantitation.

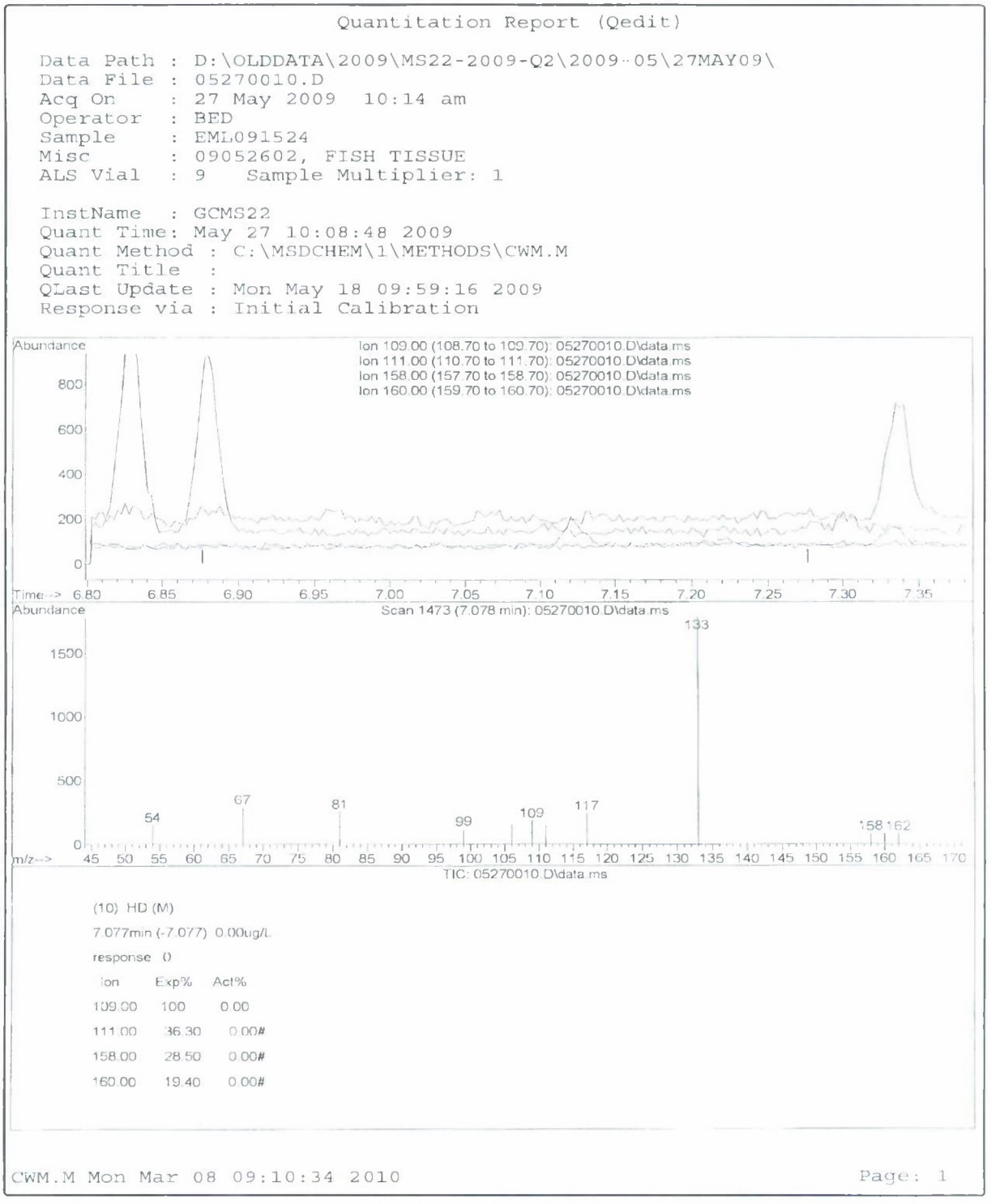
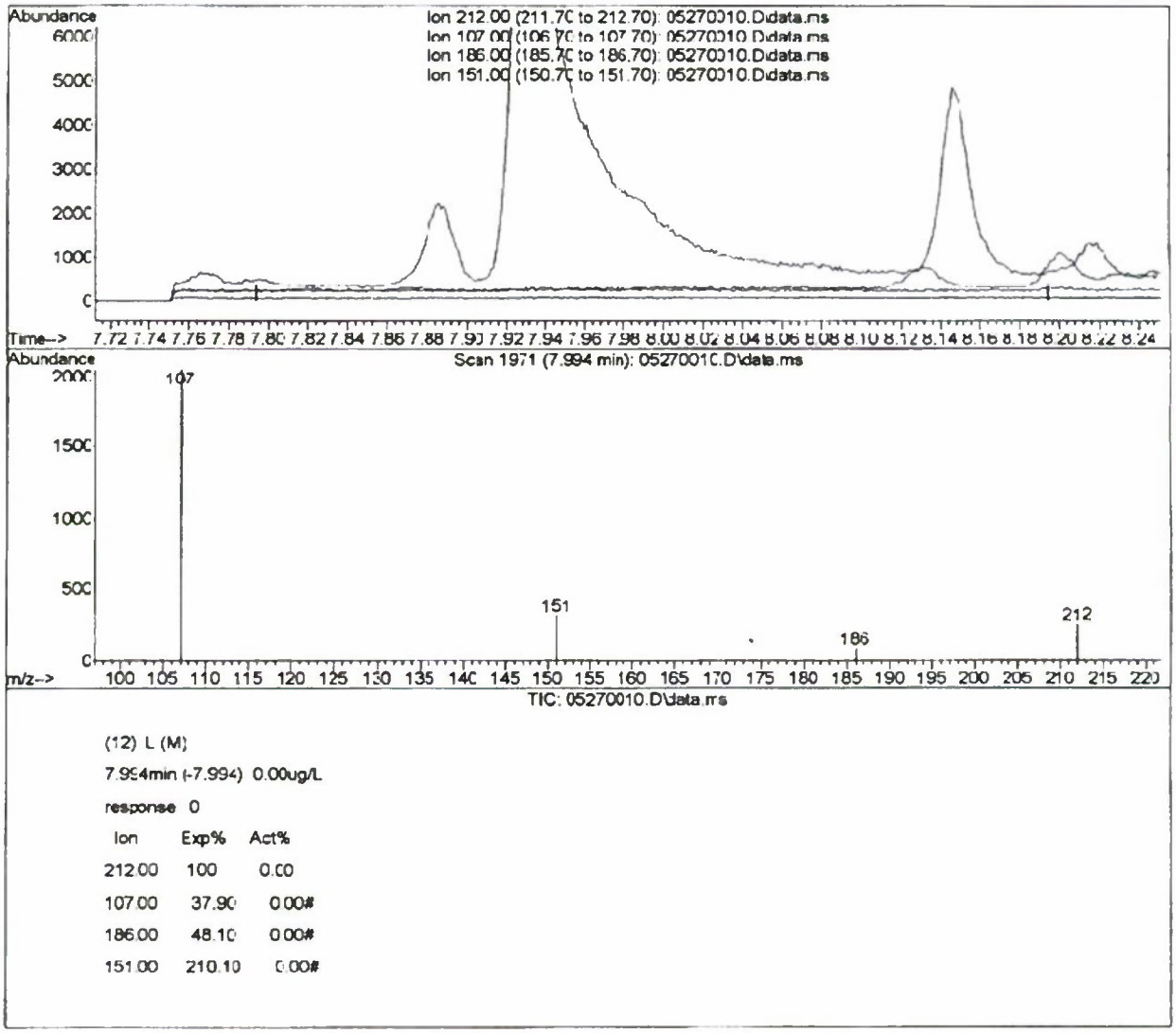


Figure 17. Sulfur Mustard (HD) EIC for Sample EML091524 (HUM001S). The speetrum shows no peaks present at the expected retention time of 7.077 min for the eharaeteristie ions. Per the IOP, this sample is considered to be elear for HD to the Limit of Quantitation.

Quantitation Report (Qedit)

Data Path : D:\OLDDATA\2009\MS22-2009-Q2\2009-05\27MAY09\
Data File : 05270010.D
Acq On : 27 May 2009 10:14 am
Operator : BED
Sample : EML091524
Misc : 09052602, FISH TISSUE
ALS Vial : 9 Sample Multiplier: 1

InstName : GCMS22
Quant Time: May 27 10:08:48 2009
Quant Method : C:\MSDCHEM\1\METHODS\CWM.M
Quant Title :
QLast Update : Mon May 18 09:59:16 2009
Response via : Initial Calibration



(12) L (M)

7.994min (-7.994) 0.00ug/L

response 0

Ion	Exp%	Act%
212.00	100	0.00
107.00	37.90	0.00#
186.00	48.10	0.00#
151.00	210.10	0.00#

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Figure 18. Lewisite Extracted Ion Chromatogram (EIC) for Sample EML091524 (HUM001S). The speetrum shows no peaks present at the expeeted retention time of 7.994 min for the characteristie ions. Per the IOP, this sample is considered to be elear for Lewisite to the Limit of Quantitation.